APPLIED AND ENVIRONMENTAL MICROBIOLOGY, June 1997, p. 2355–2360 Vol. 63, No. 6
0099-2240/97/$04.00+0

Modelling the Growth Rate of *Escherichia coli* as a Function of pH and Lactic Acid Concentration

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Received 12 November 1996/Accepted 1 April 1997

The growth rate responses of *Escherichia coli* M23 (a nonpathogenic strain) to suboptimal pH and lactic acid concentration were determined. Growth rates were measured turbidimetrically at 20°C in the range of pH 2.71 to 8.45. The total concentration of lactic acid was fixed at specific values, and the pH was varied by the addition of a strong acid (hydrochloric) or base (sodium hydroxide) to enable the determination of undissociated and dissociated lactic acid concentrations under each condition. In the absence of lactic acid, *E. coli* grew at pH 4.0 but not at pH 3.7 and was unable to grow in the presence of ≥8.32 mM undissociated lactic acid. Growth rate was linearly related to hydrogen ion concentration in the absence of lactic acid. In the range 0 to 100 mM lactic acid, growth rate was also linearly related to undissociated lactic acid concentration. A mathematical model to describe these observations was developed based on a Bělehrádek-like model for the effects of water activity and temperature. This model was expanded to describe the effects of pH and lactic acid by the inclusion of novel terms for the inhibition due to the presence of hydrogen ions, undissociated lactic acid, and dissociated lactic acid species. Preliminary data obtained for 200 and 500 mM total lactic acid concentrations show that the response to very high lactic acid concentrations was less well described by the model. However, for 0 to 100 mM lactic acid, the model described well the qualitative and quantitative features of the response.

Predictive microbiology combines knowledge of bacterial growth responses over a range of conditions with the power of mathematical modelling to enable predictions of growth. By use of this technique, questions about microbial food spoilage and food safety may be answered by objective analysis based on scientific knowledge. This is especially relevant in light of the increasing incidence of food poisoning in many countries (6). *Escherichia coli* has risen to prominence as a food-poisoning organism due to well-documented and publicized outbreaks of the enterohemorrhagic strain O157:H7. It is considered an important pathogen due to evidence of its low infective dose and the serious illnesses it can cause in young children (15).

Food-manufacturing processes that decrease the pHs of foods and produce organic acids, for example, pickling or fermentation, are extensively used as mechanisms of preventing microbial growth in foods and of ensuring food safety. However, recent reports have described tolerance of pathogenic *E. coli* strains for low pH (14), survival in foods of low pH (37), and resistance to the lethal effects of very low pH (20). Therefore, it is important to understand and to be able to predict the pH responses of microorganisms so as to determine as accurately as possible the risks in different foods and the stringency of environmental conditions necessary to reduce or control their growth.

This paper describes an investigation of the effects of suboptimal pH and lactic acid on the growth rates and limits of a nonpathogenic strain of *E. coli* and the evaluation of a theoretical model to describe that behavior.

MODEL DEVELOPMENT

This model was developed for application to food in which the pH was generally in the range of acidic to neutral. Therefore, only suboptimal pH (taken to be a pH of less than 7) was considered for modelling, although data for values extending to pH 8.45 were collected.

Suboptimal pH term. Baranyi et al. (7) considered it desirable that models should embody the known or assumed qualitative features of the phenomenon being modelled. It has been observed (18) that the pH responses of microorganisms consist of a plateau of constant growth rates over a range near the optimal and a more rapid decline in the growth rate as pH is reduced, until no growth is observed. However, Cole et al. (11) reported that the growth rate response is directly proportional to the hydrogen ion concentration $[H^+]$. In the suboptimal pH range, both observations are satisfied by the expression resulting from the following considerations.

We assume that the growth rate is proportional to the amount by which $[H^+]$ is less than the maximum value which prohibits growth. Then let

$$\text{rate} = c \cdot ([H^+_{max}] - [H^+]) \quad (1)$$

where c is a constant of proportionality, and $[H^+{}_{max}]$ is the theoretical maximum $[H^+]$ beyond which no growth is possible, and which is assumed to be constant for a given strain of bacteria.

Let pH_{min} be the pH corresponding to $[H^+{}_{max}]$. Since, by definition, $pH = -\log_{10}[H^+]$, equation 1 can be rewritten as

$$\text{rate} = c \cdot (10^{-pH_{min}} - 10^{-pH}) \quad (2)$$

$$\text{rate} = (c \cdot 10^{-pH_{min}}) \frac{(10^{-pH_{min}} - 10^{-pH})}{10^{-pH_{min}}} \quad (3)$$

Let $b = c \cdot 10^{-pH_{min}}$. Substituting for c and rearranging gives

$$\text{rate} = b \cdot \left(1 - \frac{10^{-pH}}{10^{-pH_{min}}}\right) \quad (4)$$

$$\text{rate} = b \cdot \left(1 - \frac{10^{pH_{min}}}{10^{pH}}\right) \quad (5)$$

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This is the pH term used in the model and embodies our original hypotheses regarding the qualitative features of the response.

Undissociated organic acid term. The Henderson-Hasselbalch equation relates the proportion of undissociated and dissociated forms of organic acid to pH and pK_a according to the following expression:

$$\frac{[A^-]}{[HA]} = 10^{pH - pK_a} \quad (6)$$

where [HA] is the concentration of undissociated form of the acid, $[A^-]$ is the concentration of dissociated (ionized) form of the acid, and pK_a is the pH at which the concentrations of the two forms are equal. Let [LAC] be the total concentration of lactic acid; therefore, since $[LAC] = [A^-] + [HA]$, then $[A^-] = [LAC] - [HA]$. Substituting for $[A^-]$ in equation 6 gives

$$\frac{[LAC] - [HA]}{[HA]} = 10^{pH - pK_a} \quad (7)$$

Cross-multiplying and rearranging to solve for [HA] gives

$$[LAC] = 10^{pH - pK_a}[HA] + [HA] \quad (8)$$

$$[HA] = \frac{[LAC]}{(1 + 10^{pH - pK_a})} \quad (9)$$

Eklund (12) has reported that both the dissociated and the undissociated forms of organic acids have inhibitory effects on bacterial growth but that the undissociated form is more inhibitory, per mole, than the dissociated form. Based on previous observations (1, 24, 29), we hypothesize that (i) there are growth-preventing concentrations of both the dissociated and the undissociated forms, (ii) growth rate is proportional to both the undissociated and the dissociated forms at less than those minimum inhibitory levels, and (iii) the effects of both forms are additive, not synergistic. Thus, we develop the following terms to model the effects of organic acid species on growth rate.

For the undissociated form, we assume that the growth rate is proportional to the amount by which the concentration of the undissociated form is less than the minimum concentration which prevents growth. Let $[U_{min}]$ be the theoretical minimum concentration of undissociated acid which completely prevents growth and which is assumed to be constant for a given strain of bacteria. Thus, we assume

$$\text{rate} = c' \cdot ([U_{min}] - [HA]) \quad (10)$$

where c' is a constant of proportionality, [HA] is as previously defined, and $[U_{min}]$ is the minimum [HA] which prevents growth (as defined above). Therefore, substituting equation 9 into equation 10,

$$\text{rate} = c' \cdot \left(\left[U_{min}] - \frac{[LAC]}{(1 + 10^{pH - pK_a})} \right) \right. \quad (11)$$

Let $m = c' \cdot [U_{min}]$; then, after rearrangement,

$$\text{rate} = m \cdot \left(1 - \frac{[LAC]}{[U_{min}] \cdot (1 + 10^{pH - pK_a})}\right) \quad (12)$$

Dissociated organic acid term. In a manner similar to that given above, we can generate a term for the hypothesized effect of dissociated organic acid on growth rate in the following form:

$$\text{rate} = p \cdot \left(1 - \frac{[LAC]}{[D_{min}] \cdot (1 + 10^{pK_a - pH})}\right) \quad (13)$$

where p is a constant of proportionality, and $[D_{min}]$ is the theoretical minimum concentration of dissociated acid which completely prevents growth and which is assumed to be constant for a given strain of bacteria (other terms are as previously defined).

Model. We have shown in the preceding development our hypotheses regarding the effects of pH and organic acids on bacterial growth rate. Combining these terms, the overall form of the model is

$$\text{rate} = c'' \cdot \left(1 - \frac{10^{pH_{min}}}{10^{pH}}\right) \cdot \left(1 - \frac{[LAC]}{[U_{min}] \cdot (1 + 10^{pH - pK_a})}\right) \cdot \left(1 - \frac{[LAC]}{[D_{min}] \cdot (1 + 10^{pK_a - pH})}\right) \quad (14)$$

where c'' is a constant of proportionality and the other terms are as previously defined.

Temperature and water activity effects. Addition of lactic acid affects water activity (32), and temperature typically fluctuates during storage and distribution of foods. For these reasons, terms for water activity and temperature effects on bacterial growth rate after the manner of McMeekin et al. (22) were included in the model.

Taking the square root of growth rate to homogenize the variance of the growth rate (26), the final model form is

$$\sqrt{\text{rate}} = C \cdot \sqrt{(a_w - a_{w_{min}})} \cdot (T - T_{min}) \cdot \left(\sqrt{1 - \frac{10^{pH_{min}}}{10^{pH}}} \cdot \sqrt{1 - \frac{[LAC]}{[U_{min}] \cdot (1 + 10^{pH - pK_a})}} \cdot \sqrt{1 - \frac{[LAC]}{[D_{min}] \cdot (1 + 10^{pK_a - pH})}} \right) + e \quad (15)$$

where C is a constant of proportionality, a_w is water activity, $a_{w\text{min}}$ is a theoretical minimum water activity for growth, T is temperature (in degrees Celsius), T_{min} is a notional value of temperature (in degrees Celsius) at which growth rate is zero, e is the error term, and other terms are as previously defined.

MATERIALS AND METHODS

Organism. *E. coli* M23 (a nonpathogenic laboratory strain) was obtained from the Department of Agricultural Science Culture Collection, University of Tasmania, Tasmania, Australia. Eosin methylene blue agar (Levine) (Oxoid CM69) was used to identify *E. coli* presumptively by colony morphology.

Growth medium preparation. Culture media were prepared to cover the suboptimal pH range by adding 88% lactic acid (wt/wt) (Univar; AR. Ajax Chemicals, Auburn, New South Wales, Australia) to overstrength nutrient broth (Oxoid CM1), which was then autoclaved. For each total lactic acid concentration, half of the broth (by weight) was then aseptically adjusted to pH 3 and half was adjusted to pH 8.5 by using NaOH and HCl solutions of various concentrations. The broths were made up to their final volumes with sterile distilled water. pH was measured with a portable meter (model 250A; Orion Research, Inc.) with a calomel-sealed flat-tip probe (Activon; AEP433). Different proportions of the two broths of different pHs (to a total of 15 ml) were mixed in L-shaped spectrophotometer tubes (L tubes) according to calibration curves to give set pH values. After vortex mixing, a 0.5-ml aliquot of the medium in each L tube was aseptically removed to determine the pH. In one set of experiments, the pH of each of 10 broths was adjusted individually, and then the broth was filter sterilized (0.45-μm-pore-size filter; sterile, 25-mm filter units; Activon). The water activity of each broth was measured with an Aqualab CX2 (Decagon Devices) dew point instrument.

Experimental conditions. The experiment was designed to examine in fine detail the responses to pH and lactic acid. A full factoral set of all of the conditions was not used; instead, only many different conditions of pHs and acid concentrations were examined. Temperature was not deliberately varied but was found to vary slightly; each L tube was kept at a constant temperature (±0.5°C)

in the range 20 to 22°C. Water activity was found to be decreased by lactic acid, the amount depending on the concentration. Temperature and water activity have each been successfully modelled (22), so that the small variations in these factors were accounted for by terms from models already created. By this approach, the model generated is specifically limited in its application to different temperatures and water activities. However, this model achieves a detailed and comprehensive description of pH and lactic acid inhibition.

Specific data points were not replicated; however, each experiment involving a different lactic acid concentration was repeated so that the data set for each concentration was taken from more than one experiment (with the exception of 25 mM). The L tubes were placed in a shaking incubator (model TN3; Advantec; Toyi Roshi International) in a constant temperature room (20 ± 1°C), which allowed as many as 60 different conditions to be tested simultaneously (usually four lactic acid concentrations at 15 different pHs for each lactic acid concentration, approximately 0.5 of a pH unit apart). L tubes were maintained for times sufficient to reveal contamination. Approximately 1% of the tubes had to be discarded. Lactic acid concentrations of 0, 25, 50, and 100 mM were used in the final model. Data for 200 and 500 mM lactic acid concentrations were also collected but were not as well described by the model.

Culture preparation. Inocula were grown in 60 ml of nutrient broth in a 200-ml conical flask, which was incubated statically at 37°C for at least 36 h prior to the commencement of the experiment. The inoculum level was chosen to give an initial percent transmittance (%T) in the L tubes of between 80 and 90. Typically, this was between 0.5 and 1 ml of the 36-h culture. %T was measured with Spectronic 20 (analog display) or 20D (digital display) spectrophotometers (Milton Roy Co.). Aliquots for pH determinations were taken aseptically from all tubes after inoculation and were used as the basis for the pH value for growth rate modelling.

Data collection. During inoculation, %T values of the cultures were measured in the L-shaped tubes at 540 nm against a sterile nutrient broth blank. Spectrophotometer calibration was checked at intervals throughout the experiments. Readings were taken at %T intervals of approximately 5 until the %T decreased to 5% or stopped decreasing. At the completion of the experiment, the temperature of each culture was measured three to five times over a period of at least 24 h with a Fluke 51K/J thermometer and a special liquid immersion probe. The averages of these readings were calculated and used in all subsequent data analyses.

Data analysis. Generation times and growth rates (1/generation time [minutes]) were calculated by fitting a modified-Gompertz function to the %T data by the method of McMeekin et al. (23). The growth rate model (equation 15) was fitted to data by using PROC NLIN (SAS Institute Inc., Cary, N.C.), a procedure for nonlinear regression modelling. A T_{min} of 4°C was derived from other modelling of *E. coli* M23 in this laboratory and was used as a constant in model fitting. This is the value obtained by other modellers for *E. coli* (30). T_{min} has been reported to be independent of pH (36). The pK_a of lactic acid at 25°C is reported to be 3.86 (10), and this value was used as a constant in fitting the model.

pK_a of lactic acid. The pK_as of lactic acid in aqueous solution and in nutrient broth were also determined by manual titration and measurement of pH at 20°C. The data were fitted by using Ultrafit 2.03 software (BIOSOFT, Cambridge, United Kingdom) to determine the pK_a.

RESULTS AND DISCUSSION

The fitted growth rate model (equation 15) is as follows:

$$\sqrt{\text{rate}} = 0.0247933 \cdot \sqrt{(a_w - 0.934)} \cdot (T - 4) \cdot \left(\sqrt{1 - \frac{10^{3.9}}{10^{\text{pH}}}} \cdot \sqrt{1 - \frac{[\text{LAC}]}{[10.7] \cdot (1 + 10^{\text{pH}-3.86})}} \cdot \sqrt{1 - \frac{[\text{LAC}]}{[823.4] \cdot (1 + 10^{3.86 - \text{pH}})}} \right) \quad (16)$$

where LAC, a_w, and pH are as previously defined. The predictions of equation 16 are shown in Fig. 1. Standard errors of parameter estimates and units of measurements are given in Table 1.

Bacterial growth inhibition by organic acids such as lactic acid is important in foods. Hydrochloric acid was used experimentally to determine the effect of hydrogen ion concentration based on the assumption that hydrochloric acid itself is not toxic because it dissociates completely in aqueous solution to form hydrogen and chloride ions. Organic acids also decrease the pH by the production of hydrogen ions; however, unlike strong acids, they do not completely dissociate. The presence

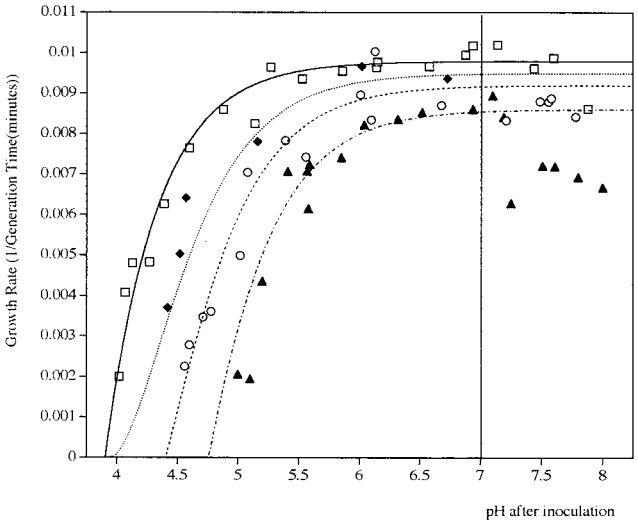

FIG. 1. Growth rate (1/generation time [in minutes]) of *E. coli* M23 in the presence of lactic acid; comparison between predictive models for 0 (solid line), 25 (dotted line), 50 (dashed line), and 100 (dashed and dotted line) mM total lactic acid concentrations and experimental data for 0 (□), 25 (◆), 50 (○), and 100 (▲) mM total lactic acid concentrations.

of the undissociated organic acid molecule is believed to cause an additional inhibitory effect (31, 32). Organic acids such as lactic acid are also able to chelate elements essential for growth, such as iron, which may be a possible mechanism of inhibition (32). It is known that lipophilic, undissociated acid molecules are able to enter the bacterial cell and once inside may dissociate (17). This is widely held to be the reason for their greater inhibition compared to that of strong acids, which can act only on the exterior of the cell. However, studies have shown that inhibition is not proportional to the pK_a and that inhibition is proportional to the undissociated acid concentration rather than the decrease in intracellular pH caused by the acid (31). This suggests that there are other inhibitory mechanisms of the undissociated acid molecule. From this knowledge, the hypothesis was formed that the concentration of undissociated lactic acid, as determined by the total lactic acid concentration and the pH, was an important inhibitory factor on microbial growth separate from but linked with the effect of pH or hydrogen ion concentration. In the present study, experiments were designed to be able to calculate the concentrations of undissociated and dissociated organic acids and thus model their effects on the growth rate of *E. coli*. These effects are reflected in the model generated and are supported by the results.

The model (equation 15) has five terms describing the inhibition due to temperature, water activity, pH, the dissociated

TABLE 1. Values of parameters of the growth rate model (equation 15)[a]

Parameter	Value (standard error)
C	0.0247933 (0.00347535)
pH_{min}	3.90 (±0.019)
U_{min} (mM)	10.7 (±0.429)
D_{min} (mM)	823.4 (±237.113)
a_{wmin}	0.934 (±0.017)
Root mean square error	0.0052083

[a] Values were estimated by fitting to the data with the SAS PROC NLIN program.

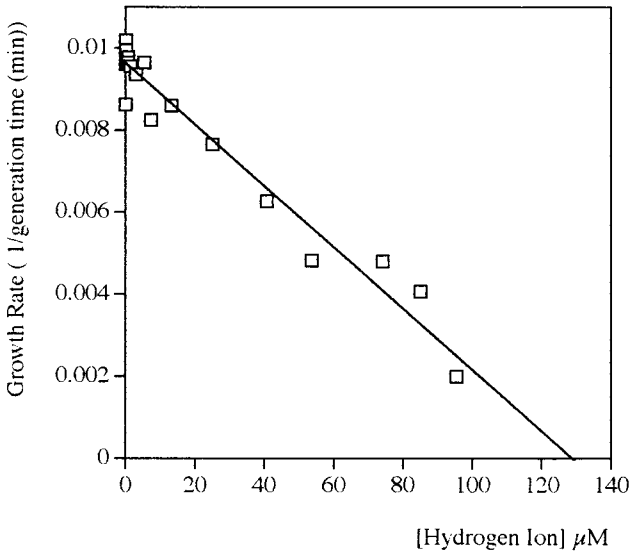

FIG. 2. Growth rate (1/generation time [in minutes]) of *E. coli* M23 in the absence of lactic acid versus hydrogen ion concentrations (micromolar). The line was obtained by linear regression.

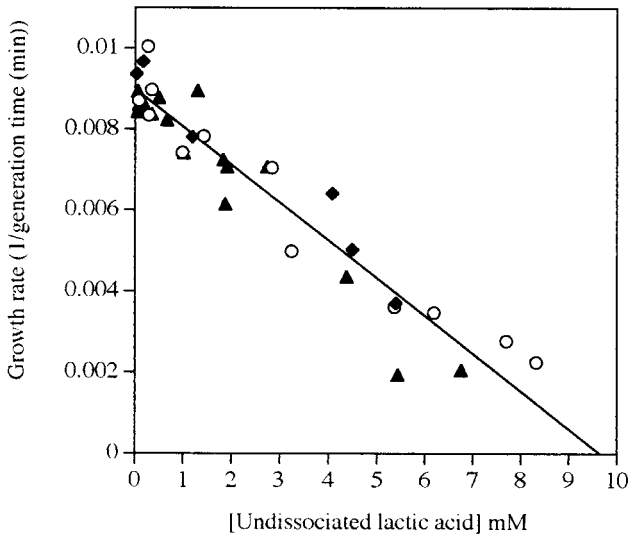

FIG. 3. Growth rate (1/generation time [in minutes]) of *E. coli* M23 versus undissociated lactic acid concentrations (millimolar) for experimental data for 25 (◆), 50 (○), and 100 (▲) mM total lactic acid concentrations. The line was obtained by linear regression.

form of the acid, and the undissociated form of the acid. The temperature and water activity terms are of the form previously used for square-root-type models (22). Although only pH and organic acid concentration were specifically under investigation, temperature and water activity varied; however, this variation was accounted for by additional terms. The water activity variation was due to the presence of lactic acid, which at high concentrations causes a significant decrease in the water activity as previously described (32).

The temperature for these experiments was intended to be as close as possible to 20°C; however, variations of as much as 2°C higher than this were observed. The $a_{w\min}$ estimate derived by fitting equation 15 was 0.934, which is similar to that found when a_w is adjusted with NaCl. NaCl and KCl have been found to have similar water activity effects (19). The water activity of broths varied from 1.000 for no added lactic acid to 0.991 ± 0.003 for 100 mM total lactic acid concentration.

The pK_a of lactic acid in complex media at 37°C was recently reported to be 3.4 (34). The actual value of the pK_a is not essential to the theoretical basis of the model, and by refitting the model using 3.4, a very similar model which fitted the data only slightly less well was obtained (results not shown). Attempts to determine the pK_a of lactic acid in the media used in this study found an average of 3.80, with a standard deviation of 0.18, although the accuracy of the method is not known. However, these studies showed no significant difference between the pK_a in aqueous solution and the pK_a in the media used, which implies that 3.86, the value for aqueous solutions (10), is an appropriate value for these media.

The pH term of this model is based on a mathematical function in which growth rate rises linearly from a threshold value to an optimum value. A linear response of growth rate to hydrogen ion concentration has been reported elsewhere (11, 29), and the observed response of growth rate to hydrogen ion concentration in this study (Fig. 2) supports the use of a linear term for the relationship. In this study, plots of growth rate versus pH (Fig. 1) show a rapid rise (usually more than about 1 pH unit) toward an asymptote. Several other studies have also reported that decreases in pH cause only relatively small decreases in the growth rate, except when the limiting pH is approached, at which a more significant decrease in the growth rate occurs (11, 29). The pH response has been described by other authors as a symmetrical parabolic curve (30, 36); however, the present study of the pH response based on a large number of closely spaced data points along the pH scale has shown that the pH response is able to be accurately described by equation 15, in which pH appears in three separate terms.

Organic acids have been found to be more inhibitory than hydrochloric acid (17). As in the present study, Glass et al. (14) found an increase in the inhibitory pH when lactic acid was the acidulant compared to hydrochloric acid. However, it was not possible to calculate the concentrations of each form of lactic acid in these experiments, because variable amounts of lactic acid were used to adjust the pH, and these quantities were not reported. Another study that separated the effects of pH and organic acid on *Yersinia enterocolitica* found that as the total concentration of lactic acid increased, there was an increase in the pH at which complete inhibition of growth occurred (9), as shown in the present study. *E. coli* O157:H7 has been found to grow on foods with pHs of greater than 4 (for example, fruit, vegetables, and beef [2, 3, 28]), which is in agreement with this model. *E. coli* O157:H7 has been found not to proliferate in foods with pHs of less than 4 (for example, mayonnaise [37] and apple cider [8]).

The present study has found that inhibition of the growth rate was proportional to the concentration of undissociated lactic acid (Fig. 3). For total lactic acid concentrations of 0 to 100 mM, growth rate inhibition was equal for equal undissociated lactic acid concentrations, regardless of the pH or total lactic acid concentration. Complete inhibition of growth occurred consistently at approximately 10 mM undissociated lactic acid for total lactic acid concentrations of 25 to 100 mM. This implies that the most significant inhibitory factor under the conditions tested was the undissociated acid. Other studies that have calculated minimum inhibitory values for undissociated and dissociated organic acids have shown values similar to those found in the present study (Table 2). As Table 2 shows, the inhibitory effect of the dissociated form of the acid is

TABLE 2. Experimentally determined values for MICs of undissociated and dissociated organic acids

Organism	Acid type	MIC_u[a]	MIC_d[b]	Reference or source
E. coli M23	Lactic	8.32		This study
Y. enterocolitica	Lactic	5–10		9
E. coli	Propionic	70	800	12
Staphylococcus aureus	Propionic	19	830	12
Bacillus cereus	Propionic	17	380	12
E. coli	Sorbic	1	100	12
E. coli	Sorbic	1	350	27[c]
Staphylococcus aureus	Sorbic	0.6	400	12
Bacillus cereus	Sorbic	1.2	110	12
Listeria innocua	Lactic (sodium lactate)	4.9	1,250	16

[a] MIC_u, MIC of the undissociated form of acid (micromolar).
[b] MIC_d, MIC of the dissociated form of acid (micromolar).
[c] Cited by Eklund (12).

weaker than the inhibitory effect of the undissociated form of the acid, since a higher concentration of the dissociated form is needed to cause complete inhibition. Some investigators (5, 13) do not believe that the dissociated form of the acid has a significant inhibitory effect. In the present study, the dissociated acid molecule was found to be a less important inhibitor of growth under conditions normally present in foods, that is, with low concentrations of organic acid and low pH. For example, meat contains approximately 10 to 100 mM lactic acid (calculated from data of Nassos et al. [25]) and has pH 5 to 6. However, when the total lactic acid concentration and pH are high, the inhibitory effect of dissociated acid may be significant; thus, its inclusion in the model is necessary to give the most complete description.

The proposed model for the growth rate of *E. coli* in response to pH and lactic acid provides a good description of the data (Fig. 1) with the fitted parameters (Table 1). A good fit to the data is evidenced by low standard errors for the parameter estimates and a low root mean square error (Table 1). The model accurately described the trends evident in the data; as total lactic acid concentration increases, there is a decrease in the maximum growth rate and an increase in the minimum pH at which growth occurs.

Preliminary data obtained for total lactic acid concentrations of 200 and 500 mM show that the response to very high lactic acid is not as well described by the model. The reasons for this are unknown and are the subject of further research. Also, equation 15 does not describe the inhibitory effect of high pH, which can be seen in Fig. 1 for 100 mM total lactic acid; however, this is of less relevance to foods, which rarely have alkaline pH. For completeness, a further term could be added to the model to describe the inhibitory effect of high pH. Sutherland et al. (33) compiled from the literature a data set of *E. coli* growth rates. Comparison of the predictions of equation 15 with two other models from Sutherland et al. (33) showed that equation 15 predicted with similar accuracy.

Previous models for the effect of pH on bacterial growth rate have been based on the assumption that the pH effects could be described by a term that had the same form as the term for water activity (4, 21) or temperature (35) in Bělehrádek-type models. Previous models are limited to modelling the effects of pH (30) and not the acidulent and thus are not able to describe the situation with foods such as meat, mayonnaise, or fermented products, which contain organic acids. Alternatively, the existing pH term must be changed when different acids are used (4).

New terms for the separate inhibition of the growth rate of *E. coli* due to pH and organic acids were developed based on the linear response to hydrogen ion concentration and to undissociated lactic acid concentration. These terms have been used to successfully model inhibition of the *E. coli* growth rate by lactic acid in terms of the effects of inhibition due to the lowering of the water activity, the concentration of hydrogen ions, and the concentration of the two different forms of organic acid.

ACKNOWLEDGMENT

This work was supported by the Australian Meat Research Corporation.

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